

SUMMARY

1. Samples of foetal and maternal blood collected at the moment of birth have been analysed by chemical means for total α -amino nitrogen and by paper chromatography for their content of individual amino-acids.

2. In nearly every case the foetal plasma had a higher concentration of α -amino nitrogen than the maternal. The increase involved all the amino-acids commonly found in these fluids.

3. These results are taken to support the view that the placenta acts as an amino-acid pump assisting the foetus to synthesize proteins.

4. A low foetal/maternal ratio for the plasma α -amino nitrogen occurred only in four out of nine cases. In these four cases the mother had definite toxæmia of pregnancy. It is suggested tentatively that this may indicate that some impairment of placental function occurs in toxæmia.

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Reactivation and Protection of Cytochrome Oxidase Preparations

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Working with isolated cytochrome systems Keilin & Hartree (1947*a, b*, 1949) showed that the loss of activity caused by treatments which affect the colloidal structure of the enzyme material could be counteracted and the activity restored by addition of proteins, or by otherwise causing formation of flocculent precipitates in the medium. They suggested that the loss of activity is due to reduced mutual accessibility between the components of the system. The first part of the present paper deals with that gradual drop in activity of the cytochrome oxidase system, which occurs when the oxidase preparation is stored, and it will be shown that this activity can also be restored by the addition of protein.

It was observed by Keilin & Hartree (1938, 1949) that the ability of cytochrome oxidase preparations to oxidize aromatic amines or succinate is rapidly destroyed by drying. The second part of the paper shows that the addition of protein before desiccation will greatly, and in some cases completely, protect the preparations against this inactivation.

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The activity of the oxidase system in tissue preparations is measured by the rate of oxidation of *p*-phenylenediamine in presence of added cytochrome *c*.

MATERIAL AND METHODS

Units. All Q_{O_2} values are in μ l. O_2 /mg. dry wt./hr.

Test system. The oxidation of *p*-phenylenediamine was followed at 39° in differential manometers; shaking frequency 120 times/min.; total fluid volume 3 ml. The substrate (final concn. 0.03 M) was tipped at the beginning of the measurements from a dangling cup into the main compartment. Proteins and other substances were brought in contact with the oxidase (with or without cytochrome *c*) in the main compartment at least 30 min. before the addition of the substrate. The initial velocity was obtained from the difference between the readings at 3 and 13 min. Phosphate buffer of pH 7.2 and of final concentration 0.08 M was used. In the left-hand flask a similar sample was set up, the only difference being that the oxidase preparation was boiled. By this means the autoxidation of the substrate under the conditions of the experiment was largely compensated for.

Cytochrome oxidase preparations. Two different oxidase preparations from horse-heart muscle were used: (1) the high-activity preparation (HA) according to Keilin &

Hartree (1947*a*), with the modifications of Keilin & Hartree (1949); (2) the low-activity preparation (LA) of Keilin & Hartree (1938), which differs from the HA preparation mainly in that it contains considerably more inactive material. This is due to the different acidity and different temperature during the final precipitation. In addition, preparations of the high activity type, but with increased content of inert protein, were used in certain of the experiments represented in Fig. 1. These preparations were obtained by slightly altering the final precipitation conditions towards the acid side (pH slightly less than 5.5). The preparations were preserved at 4°. For desiccation experiments they were used within 3 days of preparation. All activities are referred to a fat-free dry weight (cf. Slater, 1949*a*). For the HA preparations the maximum activity calculated for saturating concentration of cytochrome *c* was found by Slater (1948) to be around $Q_{O_2} = 3400 \mu\text{l./mg. dry wt./hr.}$ The preparations used in the present study had, when not otherwise stated, about the same activity when freshly prepared. For certain experiments with desiccated preparations the cytochrome oxidase prepared according to Haas (1943) was used.

Cytochrome *c* prepared according to Keilin & Hartree (1945), containing 0.34% Fe, was used dissolved in 0.5% (w/v) NaCl. The final NaCl concentration in the test system was adjusted in each experiment to 0.0115M by further addition of NaCl.

Desiccation. The usual method of vacuum drying was used, except that the preparation was frozen to -12° before desiccation. The dry powder was easily suspended again in water or phosphate buffer. The suspension obtained was slightly flocculent, whereas the untreated fresh preparation was a homogeneous, finely dispersed suspension.

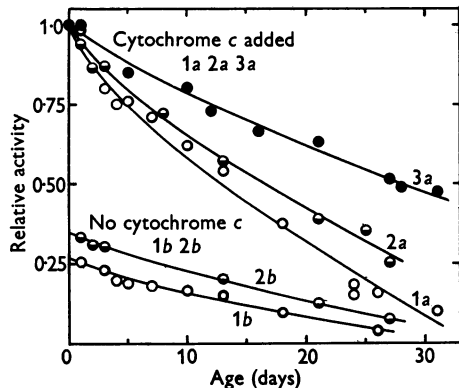


Fig. 1. Dependence of the activity of cytochrome oxidase on the age of the preparation. For purity, etc., of the preparations, cf. Table 2. Activity of fresh preparations in presence of added cytochrome *c* = 1.0. Final concentrations of added cytochrome *c*: in 1*a*, $73.5 \times 10^{-6} \text{M}$; in 2*a* and 3*a*, $38.3 \times 10^{-6} \text{M}$; in 1*b* and 2*b*, 0. Q_{O_2} of preparations: 1, 3350; 2, 2800; 3, 1150.

EXPERIMENTS AND RESULTS

Activity changes due to ageing of the preparation

The activity of the LA preparation falls gradually with increasing age of the preparation; thus Borei (1945) found that a preparation kept for 4 days at 4°

had lost 26% of its activity. The HA preparation is susceptible in the same way. The activity in the most highly purified preparations is almost completely lost in a month (Fig. 1).

The activity of aged preparations cannot be restored by the addition of cytochrome *c*. This is obvious from the values of oxidase activity at saturating cytochrome *c* concentration in preparations of different age (Table 1).

Table 1. *Activity of a high-activity preparation of cytochrome oxidase at different ages and at different concentrations of added cytochrome *c**

(The values for saturating cytochrome *c* concentration were obtained graphically according to the analysis of Lineweaver & Burk (1934). For conditions of test see text, p. 227.)

Age of preparation (days)	Activity (Q_{O_2} ; $\mu\text{l./mg. dry wt./hr.}$)		
	No cytochrome <i>c</i> added	Added cytochrome <i>c</i> $73.5 \times 10^{-6} \text{M}$ (final concentration)	Saturating cytochrome <i>c</i> concentration
1	710	2760	3000
3	640	2230	2390
7	500	1990	2260
10	470	1740	1920

A considerable bacterial flora progressively develops in preparations kept at 4°. The proteolytic and general disintegrating activities of this flora seem, however, not to be the main cause of the decrease in oxidase system activity, as Slater (1949*b*) obtained the same type of gradual inactivation in oxidase preparations preserved in presence of glycerol. That the bacterial flora in itself is not responsible for the measured activities in aged preparations is clearly proved by the curves in Fig. 1, which approach zero activity in spite of the increasing bacterial contamination.

Table 2. *Activity and cytochrome oxidase concentration of the preparations in Fig. 1*

($Q_{O_2}(\text{max.})$ signifies maximal activity of fresh preparation calculated at saturating cytochrome *c* concentration (Lineweaver & Burk, 1934) as $\mu\text{l. O}_2$ consumed/mg. fat-free dry matter/hr. Oxidase concentration expressed as $\mu\text{l. O}_2$ consumed/ml. fresh preparation/hr. at saturating cytochrome *c* concentration. For conditions of test see text, p. 227.)

Oxidase preparation	$Q_{O_2}(\text{max.})$	Dry wt. (fat free) (mg./ml.)	Oxidase concentration
1	3350	11.4	38,000
2	2800	12.2	34,000
3	1150	32.5	37,000

The higher the purity (Q_{O_2} at saturating cytochrome *c* concentration) of the preparation, the steeper is the drop in the activity on ageing (Fig. 1). From Table 2 it is obvious that the three prepara-

tions represented in Fig. 1 contain roughly the same amount of oxidase per ml. Thus they differ mostly in their content of foreign proteins. This suggests that such proteins also have a protecting effect.

Addition of protein to fresh and to old preparations

Addition of proteins to the oxidase system (Fig. 2) has no influence on the initial rate of oxygen consumption if the oxidase is freshly prepared. It helps, however, to maintain the oxidation at a high level for a longer time. With aged oxidase the addition of protein, apart from this effect, produces a marked increase of the initial rate of oxygen uptake.

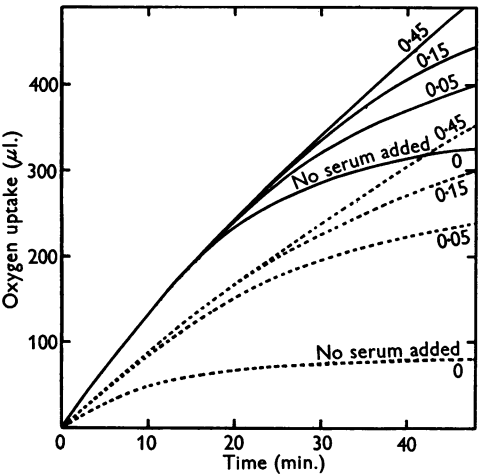


Fig. 2. Influence of addition of serum on the activity of freshly prepared and of aged high-activity preparations of cytochrome oxidase. The amount of added serum (ml.) is denoted on the curves. Final concentration of added cytochrome *c* was 38.3×10^{-6} M. — fresh preparation. - - - - 27 days old preparation.

These effects are brought about by many different proteins (Table 3) as well as by calcium chloride and to some extent by suspensions of lecithin. Plasma and serum contain, in addition to proteins, several other substances which might take part in activating the oxidase system. However, it was shown that the dialysable components of these fluids are not responsible for the activation.

In the experiments summarized in Fig. 2 addition of serum did not completely restore the activity of the aged preparation to the level of the fresh one. A complete restoration is, however, obtainable during the earlier parts of the ageing process. Thus a complete restoration is found within a period up to 10 days after preparation, during which time the oxidase activity, when measured without protein addition, drops to 60–70 % of that of the fresh preparation.

Table 3. *Augmentation of oxygen uptake in systems containing aged high-activity preparations of cytochrome oxidase*

(Final concentration of added cytochrome *c* was 38.3×10^{-6} M. For conditions of test see text, p. 227.)

Added substances (0.15 ml.)	O ₂ uptake between 3 and 13 min. reaction time relative to water control
Preparation 24 days old	
Water	1.0
Gelatin, 5% (w/v) solution	1.2
Lecithin, 5% (w/v) suspension	1.3
CaCl ₂ (0.03 M)	2.0
Denatured globin (horse), 5% (w/v) solution	2.3
Serum (horse)	2.3
Plasma (horse)	2.4
Preparation 14–17 days old	
Water	1.0
Serum (hen)	1.4
Serum (horse)	1.6

In experiments without added cytochrome *c* the effect of proteins is of the same type as in presence of added cytochrome *c*.

Effect of desiccation: addition of serum after and before desiccation

Using a slightly different procedure of desiccation from that adopted here, Keilin & Hartree (1949) found that vacuum drying decreased the succinic oxidase activity by about 50 %. The cytochrome oxidase activity of similar HA preparations decreased in the present investigation (cf. Fig. 4) by 40–60 %. LA preparations, on the other hand, were more resistant and lost only about 25 % of their activity. It was found impossible to restore the activity of desiccated preparations to the original level by addition of cytochrome *c*, as can be seen from the experiments on HA preparations in Table 4.

Table 4. *Influence of added cytochrome c on the activity of desiccated high-activity preparations of cytochrome oxidase*

(The values for saturating cytochrome *c* concentrations were obtained graphically according to Lineweaver & Burk (1934).)

	Activity (Q_{O_2})		
	No cytochrome <i>c</i> added	Added cytochrome <i>c</i> 73.5×10^{-6} M (final concentration)	Saturating cytochrome <i>c</i> concentration
Untreated preparation	640	2230	2390
Desiccated preparation I	260	890	1020
Desiccated preparation II	230	775	870

In fact, the percentage increase in activity on adding a given amount of cytochrome *c* is the same after as before desiccation.

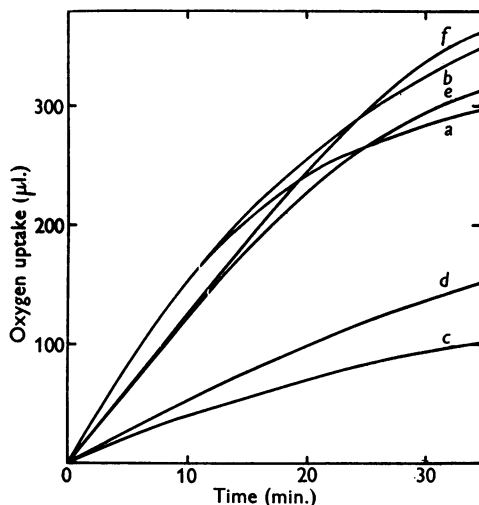


Fig. 3. Effect of desiccation in presence and in absence of serum on the activity of high-activity preparations of cytochrome oxidase. Final concentration of added cytochrome *c*, 38.3×10^{-6} M. Preparation in *e* and *f* had been desiccated together with 1 ml. horse serum/ml. *a*, untreated oxidase; *b*, as *a* + 0.15 ml. serum; *c*, dried oxidase; *d*, as *c* + 0.15 ml. serum; *e*, oxidase dried together with serum; *f*, as *e* + 0.15 ml. serum.

On the other hand, Keilin & Hartree's (1949) vacuum-dried preparations regained, on addition of cytochrome *c*, full succinic oxidase activity on addition of proteins or of certain metal salts. This

was not the case, however, with the preparations used in this investigation.

As shown in Fig. 3, addition of protein does not cause more than a slight increase in the initial oxidation rate of the desiccated preparation. However, the addition of foreign protein before desiccation of the oxidase preparation produced a most striking protective effect (Fig. 3, curve *e*), abolishing almost completely the inactivation due to desiccation.

Table 5 shows that with increasing amount of protein added before desiccation, the activity of the oxidase preparation gradually approaches the values given by undesiccated preparations to which a corresponding amount of protein has been added. With preparations desiccated together with sub-optimal amounts of protein, further addition of protein increases the activity, as shown in Fig. 3, curve *f*.

Stability of desiccated preparations

These experiments are summarized in Fig. 4. The HA preparations, represented in curves 1, 2 and 3, start immediately after desiccation with activities between 40 and 60 % of those of the untreated fresh preparations, drop considerably in activity during the next few weeks, but remain thereafter remarkably constant for long periods at a level of about 25 % of that of the original untreated preparation. It seems quite immaterial whether desiccated preparations are preserved at 4° or at room temperature, as the activity curves for these two conditions run completely parallel in an experimental series covering 1.5 months. The desiccated LA preparations behave in a somewhat similar manner (curve 4).

Table 5. Activity of preparations of cytochrome oxidase after desiccation in presence of different amounts of horse serum

(High-activity preparations; final concentration of added cytochrome *c*, 38.5×10^{-6} M.)

Preparation of oxidase	Treatment	Serum addition (ml./ml. oxidase)	Relative O ₂ uptake (as % of highest uptake)	
			3-13 min.	0-43 min.
A	Desiccated on the day of oxidase preparation. Serum added before desiccation	0	12	37
		0.02	13	44
		0.2	14.5	51
		0.4	19.5	64
		0.8	23	74
A	Untreated, fresh. Serum added in the test	0	30	88
		2.5	30	100
B	Desiccated 3 days after the oxidase preparation. Serum added before desiccation	0	14.5	47
		0.25	29	74
		0.5	30	80
		1.0	30.5	85
		2.0	31	95
		4.0	31.5	99
B	Untreated, 3 days old. Serum added in the test	0	29	73
		1.25	32	90
		3.75	33	100

As shown in the previous section, the HA preparations retain their original activity more or less completely on desiccation in presence of added protein (horse serum). Their loss of activity on keeping (curves 1s and 2s) is slower than that of a preparation dried in absence of added protein.

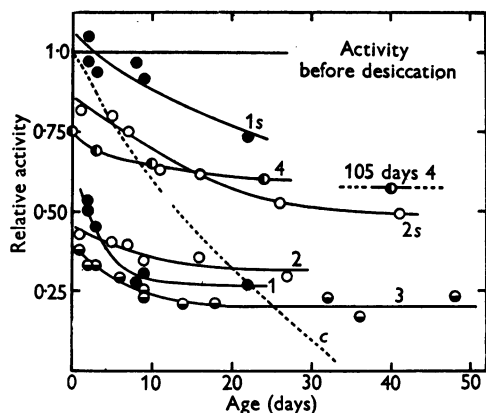


Fig. 4. Dependence on age of the activity of untreated and of desiccated preparations of cytochrome oxidase. *C*, high-activity preparation, untreated. 1, 2 and 3, the same, desiccated. 4, low-activity preparation, desiccated. 1s and 2s, preparations 1 and 2 desiccated in the presence of 1.0 and 0.4 ml. horse serum/ml. respectively. Final concentration of added cytochrome *c*; in *C* and 3, $73.5 \times 10^{-6} \text{ M}$; in 1, 1s, 2 and 2s, $38.3 \times 10^{-6} \text{ M}$; in 4, $32.5 \times 10^{-6} \text{ M}$. All activities refer to the activity of the untreated preparation on the day of desiccation (relative activity, 1.0).

The cytochrome oxidase prepared according to Haas (1943) keeps well at 4° and seems (probably owing to its higher pH) to be a less suitable substrate for bacteria. Thus after 12 days at 4° the activity was found to be 97 % of the original. Even after a month the activity was still quite considerable and no bacterial contamination could be detected (cf. Borei, 1945). The preparation is, however, very susceptible to any cold treatment. Thus a simple freezing at -12° and subsequent thawing at room temperature diminished the activity to 57 % of the original and on keeping for 12 days at 4° the activity was down to 22 % of the original. This susceptibility to freezing is in contrast with the properties of the Keilin & Hartree preparations, used in the present investigation, which stand such cold treatment without marked loss of activity. Desiccation, which involves exposure to low temperature, is thus very damaging to the Haas (1943) preparation: only between 10 and 20 % of the original activity remains after such treatment. Moreover, desiccation aggregates the preparation and makes it very viscous.

An impairment of the accessibility of the endogenous cytochrome *c* results from cooling of the preparations with solid CO_2 in acetone or with liquid

air (Keilin & Hartree, 1938, 1940). In the present investigation the preparations were invariably pre-cooled to -12° in order to avoid temperature denaturation during desiccation. It was found, however, that even repeated freezing at -12° and thawing at room temperature did not alter the accessibility of the endogenous cytochrome *c*. Freezing at lower temperatures (-70° or -183°), on the other hand, had a marked effect even with a single treatment.

DISCUSSION

The experiments presented here show that the activity of the cytochrome oxidase system, which gradually decreases during ageing of the oxidase preparation, can be restored by the addition of protein. This re-activation is complete only during the earlier phase of the ageing.

Keilin & Hartree (1947b, 1949) found that the impairment of the activity of the succinic oxidase system (of which the cytochrome oxidase system is a part), which is brought about by treatment such as vacuum drying, repeated freezing at low temperatures, or suboptimal phosphate concentration, can be restored by the addition of protein. In a similar way they explained the decrease in the activity of the cytochrome oxidase system in a preparation which had been fractionated by ultracentrifugation. They suggested that the effects of various treatments were due to changes in the colloidal structure of the preparations impairing the mutual accessibility of the components of the system. The re-activation of these systems on addition of proteins or other substances can, according to them, be explained by re-establishment of a suitable colloidal structure in which the different components regain an orientation which leads to greater mutual accessibility.

The results of the present investigation point to a similar consideration in the case of the decrease in the activity of the ageing oxidase preparation and in the re-activation of the aged preparation through the addition of protein. It thus follows that a proper colloidal arrangement is essential not only for the function of the entire succinic oxidase system, but also for that part of it which may be termed the cytochrome oxidase system which comprises, as far as is now known, cytochromes a_3 , *a* and *c*.

In the re-activation experiments, added proteins influence the activity in two ways: by increasing the initial rate of substrate oxidation, an effect which is marked in aged preparations but absent in fresh ones, and by a general maintenance of the substrate oxidation for a longer time both in fresh and in old preparations.

Keilin & Hartree (1949) stress the significance of the phosphate concentration for the optimal activity of the succinic oxidase system. They showed that the

effect of the addition of protein to a fresh preparation at suboptimal phosphate concentration is to raise its activity to the optimal level. In the present investigation no increase in the activity could be observed on adding protein to the fresh oxidase preparations. This must mean that the conditions were optimal, though the phosphate concentration used (0.08 M) is lower than 0.15 M, which was found to be optimal for the succinic oxidase system.

In addition to a re-activating effect of protein on oxidase preparations after their activity has been impaired, protein may also exert a protective function if it is added before the preparation is subjected to such activity-impairing influences. In fact it was clearly demonstrated that the addition of protein not only ensures complete protection against the adverse effects of desiccation, but also confers a greater stability on the dried material.

The cause of inactivation by desiccation is thought likewise to be an alteration of the colloidal structure of the preparation, leading to an impairment of the accessibility of the components of the system.

From the above considerations it is conceivable that the two effects of the addition of proteins, i.e. the re-establishing of an impaired activity, and the protection against loss of activity, may be brought about by the same mechanism. First, if the catalysts become disarranged, then they may be brought back to a favourable arrangement (not necessarily the original one) by the added inert proteins providing new 'points of attachment'. Secondly, if the presence of inert proteins protects the catalysts against colloidal disarrangement then this may occur by providing points of attachment for the formation of favourably arranged and stable catalyst complexes.

The gradual drop in activity of untreated oxidase preparations and the rapid development of a bacterial flora are disadvantages when utilizing the preparations for biochemical measurements. The use of such preparations in enzymic experiments is thus in most cases limited to the first few days after preparation. Desiccation checks the development of the bacteria, but the simultaneous drop in oxidase activity severely limits the usefulness of the preparation. Desiccation in the presence of inert protein, however, provides a means to retain the activity, and to prevent its subsequent rapid decrease. Thus this type of oxidase preparation can with advantage be utilized over a period of at least a month, whereas the untreated preparation starts to putrefy after a few days even if kept in the refrigerator.

SUMMARY

1. The activity of the oxidase system in Keilin & Hartree's (1947 *a*, 1949) heart-muscle preparation decreases gradually with increasing age of the preparation. The higher the activity of the fresh preparation the steeper is its decrease on ageing. After about 30 days such preparations become practically inactive. Addition of cytochrome *c* alone cannot restore the activity of the aged preparations. The decrease in the activity is considered to be caused by alterations in the colloidal structure of the oxidase preparation, impairing the mutual accessibility of its components.

2. Addition of proteins or certain other substances raises the activity of aged preparations, but not of fresh ones. In the earlier stages of the ageing process, the increase results in a complete restoration of the activity. The effect is considered to be due to influences on the colloidal structure of the oxidase preparation, resulting in a re-establishing of the mutual accessibility of its components.

3. In older preparations (aged 10 days or more) the loss of activity gradually becomes irreversible.

4. Desiccation of the preparation greatly impairs its activity, but preparations of low activity are more resistant than those of high activity. The activity cannot be restored by cytochrome *c*, and the addition of serum has only a very slight effect. The impairment of the activity of the system on desiccation is considered to be caused by a colloidal disorientation of the components of the catalyst complexes.

5. If the desiccation is carried out in presence of a sufficient amount of serum, the activity remains unaffected.

6. The desiccated preparations drop considerably in activity during the initial period after desiccation, but gradually reach a constant level. If the preparations are desiccated together with serum the drop is markedly slower.

7. On account of the retained activity, and its slower decrease as well as the absence of bacterial development, the preparations desiccated together with protein have a considerable advantage over the untreated preparations kept at 4°, since the period over which a preparation may be used is extended from a few days to at least a month.

It is the pleasant task of the author to thank Prof. D. Keilin, F.R.S., and Dr E. F. Hartree for their advice and criticism. The experiments on the stability of the desiccated preparations have in part been carried out by Dr S. Renvall, for which collaboration the author is much indebted.

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Extrahepatic Lipid Synthesis

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It is generally thought that in the animal body the liver is the principal organ concerned with both the synthesis and degradation of fatty substances. Except for a recent article by Masoro, Chaikoff & Dauben (1949) there is no definite evidence that fatty acids or cholesterol can be synthesized outside the liver in the adult animal, although Waelsch, Sperry & Stoyanoff (1940b) have demonstrated the synthesis of fatty acids and cholesterol in the brain of growing rats during myelination. Masoro *et al.* (1949) noted the appearance of ^{14}C -labelled fatty acids after the injection of ^{14}C -labelled glucose in liverless animals whose intestinal tracts were also removed. They inferred that the conversion of carbohydrate into fat occurred probably in the adipose tissues. Srere, Chaikoff & Dauben (1948) quoted some unpublished experiments in which conversion of acetate to cholesterol was demonstrated in the liverless rat; they did not, however, state in which organ the conversion occurred.

In this article results obtained with the aid of deuterium (D) and ^{14}C -labelled acetate are recorded, and it is shown that internal organs other than the liver synthesize both fatty acids and cholesterol.

METHODS AND MATERIAL

Female rabbits (2–3 kg.) were the experimental animals. All except one were pregnant. The results obtained on the fetuses of these animals have already been recorded (Popják & Beekmans, 1949a, b; 1950).

The rabbits were given by intravenous injection an amount of pure D_2O (made isotonic with NaCl) sufficient to bring the concentration of D_2O in the body fluids to a level of 1.5%; the drinking water was then replaced by 2% D_2O in tap water. The concentration of D_2O in the body fluids was thus maintained constant at a level of about 1.5%. The animals were given the heavy water for 1–12 days. It has been shown by the extensive studies of Schoenheimer and his collaborators and others (see Schoenheimer, 1941),

that the rate of incorporation of D from the body water into fatty acids and cholesterol under such experimental conditions may be used as a measure of the turnover of these substances in the tissues.

Na acetate labelled in the carboxyl C with ^{14}C was injected intravenously, two or three injections being given daily for 1–4 days. The total dose of ^{14}C was 50–100 μC . The specific activity of the injected acetate was 100 $\mu\text{C}/8.6$ mg. of anhydrous Na acetate.

The animals, under nembutal-ether anaesthesia, were killed by bleeding from the aorta. The blood was heparinized. The D and ^{14}C contents of the fatty acids and cholesterol were examined in the liver, plasma, gastro-intestinal tract, lungs, kidneys, spleen, heart, skeletal muscle, adrenals, ovaries and brain. The gastro-intestinal tract was washed clean with warm water before extraction. Data will be presented only for those organs in which the isotope concentrations of the lipids exceeded those found in the liver, as this proves extrahepatic synthesis. All experimental evidence tends to show that plasma lipids are derived from the liver (see Discussion, p. 236); therefore an isotope concentration higher in tissue lipids than in plasma lipids may also be regarded as a proof of the extrahepatic origin of the former.

The methods of extraction and fractionation of lipids and the methods of isotope determinations were those already described (Popják & Beekmans, 1950). In the one experiment, in which the free and esterified cholesterol were examined separately (see Table 2), the free cholesterol was precipitated as the digitonide from the acetone supernatant obtained after the precipitation of phospholipins. After removal of the free cholesterol as the digitonide and of the excess digitonin, the solution containing glycerides and cholesteryl esters was saponified. The unsaponifiable matter was separated from the soaps by extraction with light petroleum (b.p. 40–60°) and the cholesterol from the esters precipitated as the digitonide.

The D content of the substances is expressed as a percentage of that found in the body water. The ^{14}C content is given in the Tables as $\mu\text{C}/\text{mg. substance}$. Under our conditions of radioactive assay a substance which contained 1×10^{-3} $\mu\text{C}/\text{mg.}$ gave 1800 counts/min. in an 'infinite thickness' sample of area 2 sq.cm.